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Review

Phosphatidylinositol 4-kinases

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Contents

1. Introduction

Inositol phospholipids were recognized very early as components of biological membranes, first in mycobacteria [1,2] and later in the mammalian brain [3] and in plants [4]. The elegant early studies of Folch revealed the existence of an inositol-containing lipid fraction in ethanol-insoluble lipid extracts of bovine brain that contained phosphate in a ratio of 2:1 to

Abbreviations: PtdIns, phosphatidylinositol; PtdIns(4)P, phosphatidylinositol 4-monophosphate; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; Ins(1,4,5)P_3, inositol 1,4,5-trisphosphate; PLC, phosphoinositidase C; DAG, diacylglycerol; PKC, protein kinase C; GPCR, G protein-coupled receptors; WT, Wortmannin

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inositol [5]. Subsequent studies identified these lipids in other tissues and clarified their structure as phosphatidylinositols containing phosphomonoester groups on their inositol rings [6]. However, the function of these then novel lipids remained unknown for some time.

Attention to these molecules was greatly increased when the Hokins described the rapid ^{32}P-labeling of phosphatidic acid and phosphatidylinositol (PtdIns) in response to stimulation by secretagogues [7], and when Michell proposed a model in which phosphatidylinositol breakdown was viewed as an early signaling event linking activation of certain cell surface receptors to calcium-regulated intracellular responses [8]. The link between phosphoinositide breakdown and the generation of an intracellular Ca^{2+} signal was uncovered when PtdIns(4,5)P_2 was identified as the primary substrate of receptor-regulated phospholipase C (PLC) [9,10], and the water soluble product of this reaction, Ins(1,4,5)P_3, was found to release Ca^{2+} from intracellular stores [11].

The phosphorylation of PtdIns to yield PtdIns(4)P and PtdIns(4,5)P_2, and the kinases catalyzing these reactions, were then logically considered as part of the metabolic sequence by which Ins(1,4,5)P_3 was generated. For a time it appeared that the function of inositol lipids had been fully explored and understood. Only recently have we begun to realize that phosphoinositides and the phosphorylation of PtdIns may have more complex regulatory functions than previously believed, and that these may reach well beyond calcium signaling. This review summarizes our current knowledge of one of the groups of phosphoinositide kinases, the PtdIns 4-kinase enzymes, and presents some ideas for future research directions concerning their versatile biological functions. Additional reviews with detailed coverage of the biochemistry of PtdIns 4-kinases are available elsewhere [12,13].

2. Biochemical studies on PtdIns 4-kinases

The early stones of PtdIns kinase research were laid in the late 1960s when enzymatic activities capable of phosphorylating PtdIns in the presence of ATP were described in several tissues. In the liver, this activity was found to be present in the plasma membrane and required Mg^{2+} or Mn^{2+}, but not Ca^{2+}, for optimal activity [14]. The plasma membrane localization of such activity was also found in other tissues, including the red blood cell membrane [15]. However, signs of additional PtdIns kinase activities associated with microsomes have already been noted in these early studies [16]. This plasma membrane-bound activity, termed type II PtdIns kinase, which is the most abundant PtdIns kinase in tissue homogenates, has long been believed to be the activity responsible for the synthesis of PtdIns(4)P, the substrate for subsequent phosphorylation to PtdIns(4,5)P_2 and thence hydrolysis by receptor-regulated PLC.

2.1. Type II PtdIns kinase

This enzymatic activity has been purified from various membrane sources, including the red blood cell membrane [17,18], liver [19], uterus [20], and A431 cells [21]. The activity is not present in the soluble fraction of tissue homogenates and requires detergents for extraction from membranes. Detergents also increase its activity toward an artificial substrate (PtdIns in detergent micelles). While this enzyme requires Mg^{2+} or Mn^{2+} for optimal performance, Ca^{2+} was found to inhibit its activity [18,20]. This feature did not fit well with the enzyme's putative biological role, namely to continuously provide substrate for PLC during agonist stimulation, since elevated cytosolic Ca^{2+} in such cells would tend to inhibit the enzyme at a time when its activity was most required. The type II PtdIns kinase phosphorylates PtdIns at the 4-position of the inositol ring [20], and also has the distinctive property of being renaturable from gels after SDS-PAGE [17,18]. The molecular size based on migration on SDS-PAGE varies with the various tissue preparations, but ranges between 45 and 55 kDa [13]. The native size of the protein has been difficult to assess, since it requires detergents to maintain its solubility, and conflicting estimates have been reported based on sedimentation characteristics in various detergents and density gradients [21,22]. Studies on the catalytic properties of the type II PtdIns kinase revealed relatively high affinity to ATP (with K_m in the 10–50 μM range) and sensitivity to inhibition by low concentrations of adenosine (K_i=10–70 μM) [17,18,20] (Table 1).

Table 1
Characteristics of type II and type III PtdIns 4-kinases

	Type II	Type III
M_r	45–55 kDa	110 and 210 kDa
K_m (ATP)	<100 μM	>400 μM
K_m (PI)	~ 20 μM	~ 100 μM
K_i (adenosine)	<50 μM	$>$ mM
Ca^{2+}-sensitivity	inhibits	no effect
Detergent effects	activates	activates
Wortmannin	insensitive	sensitive ($IC_{50} \sim 50$–100 nM)
LY 294002	insensitive	sensitive ($IC_{50} \sim 50$–100 μM)
Localization	membranes	soluble (partially membranes)

Although indications of the existence of other, membrane-bound PtdIns kinases had been noted in early studies, the nature of these activities was not addressed until the late 1980s. In 1988, Whitman et al. identified two types of PtdIns kinases from fibroblasts, one with the characteristics of a type II PtdIns 4-kinase, and another which was inhibited (rather than stimulated) by detergents, and was not sensitive to adenosine [23]. This latter activity, termed type I PtdIns kinase, turned out to phosphorylate PtdIns at the 3 (and not the 4) position of the inositol ring and, therefore, established a new group of enzymes, the 'PI 3-kinases'[1] [24]. Research on PI 3-kinases has exploded during the last ten years whereas, in comparison, the PI 4-kinase field has lagged behind. The exciting developments on PI 3-kinases are covered in a separate chapter and will not be further discussed here. However, another PtdIns kinase was described in 1987 when Endemann et al. reported the presence of two separable PtdIns 4-kinase activities in cholate extracts of bovine brain that were distinguished by their size and catalytic properties. While one of these activities was the type II enzyme, the other, (larger sized) activity was a novel enzyme which was subsequently termed type III PtdIns kinase [22].

2.2. Type III PtdIns kinase

This activity, isolated from cholate extracts of bovine brain, was found to have a native molecular size of 230 kDa based on sedimentation in a sucrose gradient. In addition to its larger size, it was distinguished from the type II enzyme by its lower affinity for ATP ($K_m = 700$ μM), low sensitivity to inhibition by adenosine ($K_i = >$ mM) [22], and lack of reaction with a monoclonal antibody that inhibits the type II enzyme [25]. A similar PtdIns 4-kinase activity of 200 kDa was partially purified from bovine uterus with similar catalytic properties [26]. Attempts to purify the type III PtdIns 4-kinase were not successful, and the isolation of a 80 kDa rat brain membrane-bound PtdIns 4-kinase activity (with adenosine insensitivity and catalytic properties similar to the type III enzyme) [27] resulted in the cloning of a protein that later turned out to be a long chain fatty acid CoA synthase [28].

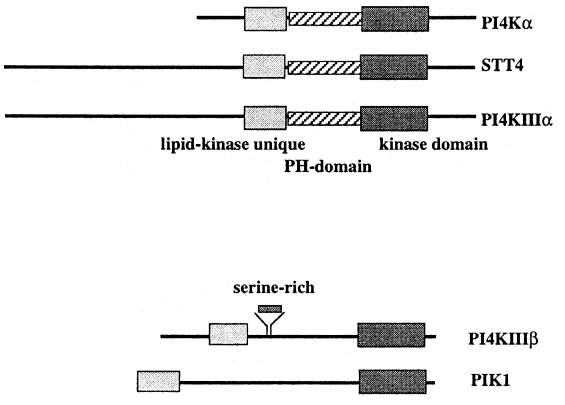
While isolation of the type III enzyme from solubilized membranes was not rewarding, a PtdIns 4-kinase activity was detected in the soluble fraction of adrenocortical homogenates with the distinctive feature of sensitivity to the PI 3-kinase inhibitors, wortmannin (WT) and LY 294002, albeit at relatively higher concentrations [29]. None of these inhibitors had any effect on the adrenal type II PtdIns 4-kinase, and the catalytic properties of this soluble activity were very similar to those of the bovine brain type III enzyme ($K_m = 400$ μM for ATP, and $K_i >$ mM for adenosine) [30]. This soluble activity was also stimulated by Mg^{2+} and Mn^{2+}, but, in contrast to the type II enzyme, was not inhibited by Ca^{2+}. Partial purification of this activity revealed two peaks on MonoQ (as well as on MonoS) columns, one with a native size of ~ 110 kDa and the other of ~ 210 kDa. These two activities had indistinguishable catalytic properties, were similarly sensitive to WT, and both gave as reaction product, PtdIns 4-phosphate [30]. In the same studies, the membrane-bound type III PtdIns 4-kinase activity of bovine brain was found to be similarly sensitive to WT, indicating that the soluble enzymes were closely related (if not identical) to the brain type III enzyme. When the two soluble type III enzymes were labeled with [^{3}H]WT (which covalently binds to the protein) and subjected to SDS-PAGE analysis (under denaturing conditions), 110 and 210 kDa bands were observed, corresponding to the native sizes of these activities. This finding revealed that these were separate entities present in soluble frac-

[1] The use of 'PI 3-kinase' is confined to the enzyme that phosphorylates PtdIns, PtdIns(4)P and PtdIns(4,5)P_2, at the 3-position, to distinguish it from PtdIns 3-kinase, the mammalian homolog of Vps34p, which only phosphorylates PtdIns.

Table 2
List of cloned type III PtdIns 4-kinases

	Human	Bovine	Rat	Dictyostelium	*S. cerevisiae*
α-forms	PI4Kα[a] (L36151) [75]	?	?	?	?
	PI4K230 (AF012872)	PI4KIIIα (U88532) [31]	230 kDa, PI 4-kinase (D83538) [76]	?	SST4 (D13717) [71]
β-forms	PI4Kβ (U81802) [78]	PI4KIIIβ (U88531) [31]	92 kDa, PI 4-kinase (D84667) [77]	DdPIK4 (U23479) [125]	PIK1 (L20220) [66]
	NPIK (AB005910) [79]				

The accession numbers and reference numbers are given in parentheses.
[a]PI4Kα was originally classified as a type II PtdIns 4-kinase.



tions as monomers [31]. These studies led to the cloning of two distinct type III PtdIns 4-kinases (see below) [31]. In the meantime, purification of the type III PtdIns 4-kinase from bovine brain as a ~210 kDa protein was also reported, as well as the isolation of cDNA clones contributing to a partial cDNA sequence [32].

At present, it is not clear why the type III PtdIns 4-kinase of bovine brain is tightly membrane-associated and the adrenocortical activity is soluble (in high salt buffer). However, we noted that although the bovine adrenal cortex does not contain significant amounts of membrane-associated type III PtdIns 4-kinase activity, the bovine brain does contain soluble activity in addition to its membrane-associated form. Moreover, although the solubilized membrane-bound activity has different chromatographic properties than the soluble counterpart, both activities label as bands of 210 kDa with [^{3}H]WT (G. Downing and T. Balla, unpublished observations). It is possible that post-translational lipid modifications make some of the enzyme more tightly membrane-bound in the brain, although splice variations (without noticeable size difference) cannot be ruled out. Further studies are required to answer this question.

In addition to these two biochemically well-characterized PtdIns 4-kinases, there are reports describing additional activities that do not clearly fit into the above classification. Saltiel et al. have purified an enzymatic activity from bovine brain myelin that was able to phosphorylate PtdIns as well as PtdIns(4)P. This PtdIns kinase activity was renatured from gels after SDS-PAGE (although its PtdIns(4)P kinase activity was not tested) and was 45 kDa in size [33]. Similarly, two membrane-bound PtdIns 4-kinase activities were purified from *Saccharomyces cerevisiae* that could be renatured from SDS-PAGE gels and were 55 and 45 kDa in size (see [34]). Both of these activities had a high K_m for ATP and low sensitivity to adenosine, although they differed in PtdIns surface concentration-dependence. Also, while the 45 kDa (but not the 55 kDa) form was inhibited by CDP-diacylglycerol [35], CTP inhibited the 55 kDa (but not the 45 kDa) activity [36]. An antibody raised against the 45 kDa form did not recognize the 55 kDa enzyme, nor did it react with the 125 kDa soluble yeast PtdIns 4-kinase (see below) [34]. These data suggest that the 45 and 55 kDa membrane-bound yeast enzymes can be separate entities and although kinetically not typical, they are probably the yeast equivalents of the mammalian type II PtdIns 4-kinase(s).

3. Cellular studies on PtdIns 4-kinases

Measurements of changes in PtdIns(4)P and PtdIns(4,5)P_2 levels in agonist-stimulated cells labeled either with [^{32}P]phosphate or *myo*-[^{3}H]inositol have allowed some important conclusions to be drawn concerning the regulation of PtdIns kinases. Although these studies suffer from uncertainties as to whether changes in radioactivity really reflect alterations in mass rather than increased specific activity of the radiolabeled phospholipid, and also that changes could represent the sum of opposing reactions (i.e. kinases and phosphatases). They, nevertheless, have the advantage that the enzymes function in their natural environment in vivo, their substrate availability and regulatory components preserved. A compromise between studies on intact cells in vivo and isolated enzymes in vitro is experiments on permeabilized cells which may help to bridge the gap between these two approaches.

It has become clear from early studies that labeling of the relatively larger pool of PtdIns by either *myo*-[^{3}H]inositol or [^{32}P]phosphate is relatively slow in resting cells and can be greatly increased by stimulation with a calcium-mobilizing agonist [37,38]. This phenomenon reflects the increased turnover of the PtdIns pool in agonist-stimulated cells. However, once labeling equilibrium is approached (although it may never be attained), following 24–48 h incubation with the radiolabel (depending on the cell type), agonist-stimulation does not cause any significant change in the level of labeled PtdIns since its conversion to polyphosphoinositides and direct breakdown by PLC is compensated by its increased synthesis (as shown for example in bovine adrenal glomerulosa cells [39]). However, when the labeling of PtdIns(4)P and PtdIns(4,5)P_2 was studied with [^{32}P]phosphate (but not with *myo*-inositol), then these minor components were labeled to a 'plateau' significantly faster (1–2 h) and to a much greater extent, than expected based on their relative abun-

dances compared to PtdIns (e.g. [40]). These data suggested that the phosphomonoester groups of PtdIns(4)P and PtdIns(4,5)P_2, (especially the 4-phosphate) has a very active turnover rate either via rapid PLC-mediated breakdown (and resynthesis from the PtdIns pool) of the small polyphosphoinositide pool, or through the action of PtdIns kinase(s) and polyphosphoinositide phosphatases. In most cells, the labeling kinetics of these phosphate groups are limited to that of the endogenous ATP pool (see [41]), and there is a relatively small contribution from the slowly labeled phosphodiester (position 1 on the inositol ring) group that reflects the specific activity of the precursor PtdIns pool. This phenomenon is important to keep in mind when analyzing labeling data and changes in inositide levels.

When labeled to near equilibrium with either *myo*-[^{3}H]inositol or [^{32}P]phosphate and stimulated with calcium-mobilizing agonists, many cells show a very rapid (within 15 s decrease in both PtdIns(4)P and PtdIns(4,5)P_2 to about 50% of their initial levels and a slower (within 10 min) return to their basal, or in some cases above basal, prestimulatory values. Comparison of the amounts of labeled inositol phosphates and the changes in lipid levels revealed that the entire labeled PtdIns(4,5)P_2 pool is turned over many times during agonist action [42]. This implies that the conversion of PtdIns to PtdIns(4)P and PtdIns(4,5)P_2 is greatly increased in agonist-stimulated cells. What was not clear, and was debated at the time, was whether the increased conversion is simply the result of the law of mass action, (i.e., that is the decreased levels of these lipids due to PLC activation per se was the cause of the increased conversion) or whether there was also a stimulation of PtdIns 4-kinase and PtdIns(4)P 5-kinase activities by some active mechanisms.

This question was easier to answer in cases of stimulation by receptor tyrosine kinases, where PtdIns(4)P levels (based on radioactivity changes) were usually greatly increased without the initial drop that was observed during stimulation via G nucleotide binding protein-coupled receptors (GPCRs) [43]. Therefore, a decreased level of PtdIns(4)P could not drive the increased PtdIns 4-kinase activity. Indeed, several studies demonstrated increased PtdIns 4-kinase activity in the plasma membrane [44] or its association with the cytoskeleton [45] or with the EGF receptor itself [21,46,47] after EGF stimulation. Characterization of this increased activity from EGF-stimulated cells led to the proposal that the activated PtdIns 4-kinase is a type II enzyme [47]. A similar conclusion was reached in studies on T-cells, where activation of CD4 by crosslinking has been shown to increase PtdIns 4-kinase activity and association of a type II PtdIns 4-kinase with CD4 [48]. These conclusions were based on enzyme assays performed after cell disruption and analysis of activity toward substrates in lipid micelles, a condition which greatly enriches and favors type II kinase activity. Nevertheless, studies on permeabilized cells using endogenous radiolabeled substrates also showed an increased PtdIns 4-kinase activity after EGF stimulation, but the identity of the kinase was not determined [49].

The possible regulation of PtdIns 4-kinase by GPCRs is more controversial. Although GTP or its non-hydrolysable analogs has been extensively studied for any ability to stimulate PtdIns 4-kinase in permeabilized cells using endogenous labeled substrates, no study has convincing evidence that PtdIns 4-kinases are activated by G-proteins [41,50]. In contrast, GTP analogs were able to increase PtdIns(4)P 5-kinase activity in permeabilized cells [41], an effect that appears to be mediated by small GTP-binding proteins [51] (see article by K.A. Hinchliffe et al., this special issue, for more details on 5-kinases). It was concluded from these studies that PtdIns 4-kinase activity is not actively regulated by GPCRs, and that this conversion step is increased solely because of falling PtdIns(4)P levels. However, our data in intact adrenal glomerulosa cells prelabeled with [^{32}P]phosphate for 4 h indicated that angiotensin II (acting on the GPCR, AT_1) rapidly increases [^{32}P]PtdIns(4)P levels if cells are depleted of Ca^{2+} and PLC activation is greatly reduced (T. Balla, unpublished observation). These data suggest that GPCRs may still be able to activate PtdIns 4-kinase activity in intact cells; the difference between the action of growth factors and GPCRs on PtdIns(4)P kinetics may simply lie in their different abilities to activate PLC.

Stimulation of PtdIns 4-kinase activity in intact cells was also observed in response to cardiotoxin [52,53] and mastoparan [54]. Although these agents also have activity in membranes and solubilized or

even in highly enriched enzyme preparations, it is not known if they have a direct action, and which type of kinase they affect, or whether they act through an endogenous mechanism (such as G-proteins). Cardiotoxin and mastoparan was shown to stimulate a plant PtdIns 4-kinase with catalytic properties of type III PtdIns 4-kinase, isolated from carrot cells [55]. The small mastoparan peptides which are also believed to activate G-proteins are basic in character, and other cationic polyamines (such as spermine and spermidine) are also able to stimulate the type II PtdIns 4-kinase [55–57]. It is not clear at present whether these phenomena have any relevance to the physiological regulation of PtdIns 4-kinase enzymes, other than the precedent that these activities can be regulated.

Regulation of PtdIns 4-kinases by cAMP-dependent pathways is controversial. In yeast, cAMP-dependent protein kinase was shown to have a positive effect on PtdIns kinase activity (it is not known whether it affected 3- or 4-kinase(s)) [58], but it was also reported that cAMP has a negative effect on yeast PtdIns kinases [59]. In purified preparations of the yeast 45 and 55 kDa membrane-bound PtdIns 4-kinases no regulation was found by the cAMP-dependent protein kinase [36]. In membranes prepared from various blood cells, PtdIns kinase activity was found to be increased by incubation with the catalytic subunit of PKA [60], but this was not reproduced when the ATP concentration was held constant [61]. In human neutrophils, the increased PtdIns(4)P levels after f-Met-Leu-Phe stimulation were prevented by preincubation with cAMP analogs [62], but this could merely be the negative effect of cAMP on PLC activation that is observed in many cells of hemopoietic origin. Altogether, it is generally agreed, that cAMP does not represent a major mechanism by which PtdIns 4-kinases are regulated.

All the above data indicate that, if any of the PtdIns 4-kinases are regulated in intact cells, they would probably be the type II enzymes. However, our data with the PI 3-kinase inhibitor, WT, suggested that this may not be the case. Initially, WT was reported to be a specific inhibitor of PI 3-kinase which did not inhibit PtdIns 4-kinase activity [63,64]. In contrast, our data on intact cells indicated that high concentrations of WT (up to 10 μM) inhibit a PtdIns 4-kinase that provides the PtdIns(4)P and PtdIns(4,5)P_2 substrates for agonist-regulated PLC activities [29]. In prelabeled cells (either [^{32}P]phosphate or [^{3}H]inositol) preincubated for 10 min with 10 μM WT, more than 60% of the labeled PtdIns(4)P is lost and the rest shows no changes in response to agonist stimulation. Basal PtdIns(4,5)P_2 levels only decrease slightly (~20%), but break down very rapidly after stimulation without being replenished. Production of labeled Ins(1,4,5)P_3 is only transient in such WT-treated cells, as is the cytosolic Ca^{2+} increase. While these observations were originally made in bovine adrenal glomerulosa cells [29], similar results have since been obtained in additional cell types and agonists (COS-1, NIH 3T3, Jurkat T-lymphoblasts, T. Balla, S. Kim, P. Varnai, unpublished observations). All of these data suggested that while relatively little PtdIns(4,5)P_2 is hydrolyzed in the absence of agonist stimulation, in the presence of an agonist, the constant supply of PtdIns(4,5)P_2 via PtdIns(4)P is provided by a PtdIns 4-kinase that is inhibited by high concentrations of WT. A Similar conclusion was reached in studies that used turkey erythrocytes [65]. These data, together with the complete insensitivity of the type II PtdIns 4-kinase to WT, indicate that the agonist-regulated production of metabolically labeled PtdIns(4,5)P_2 relies on the function of PtdIns 4-kinases that are distinct from the type II enzyme. The search for such a kinase resulted in the purification and cloning of two WT-sensitive, type III PtdIns 4-kinase enzymes [31].

4. Molecular cloning and genetics of PtdIns 4-kinases

4.1. Studies in yeast

The first cloning of a PtdIns 4-kinase was from the yeast, *Saccharomyces cerevisiae* [66] (Table 2). This enzyme, encoded by the gene *PIK1*, was previously purified to homogeneity as a 125 kDa protein from the soluble fraction of broken yeast cells [67]. The characteristics of this soluble PtdIns 4-kinase (low sensitivity to inhibition by adenosine, K_m = 100 μM for ATP) were closer to those of the type III than the type II enzyme. It is noteworthy that successful purification required the creation of a yeast strain in which one of the heat shock proteins, Hsc82, was

genetically eliminated and another, Hsp82, was suppressed [67], since these proteins (homologs of the mammalian Hsp90) were tightly associated with the PtdIns 4-kinase activity during purification. Based on peptide sequences obtained from the purified protein, the enzyme was subsequently cloned. Interestingly, the same enzyme was almost simultaneously cloned from yeast by screening an expression library with an antibody against the nuclear pore complex [68]. Protein sequence comparison between Pik1p and the then known mammalian PI 3-kinase, p110 [69] and the yeast PtdIns 3-kinase, Vps34p [70], revealed two homologous regions: the 273 residue C-terminal segment, which is about 30% identical between the proteins and corresponds to their catalytic domain, and a shorter (~60 residues) N-terminal segment (with about 25% sequence identity) located in the center of the Vps34p and p110 molecules. Genetic elimination of *PIK1* results in a lethal phenotype, indicating that the function of this enzyme is essential for survival in yeast [66,68].

Another PtdIns 4-kinase has been cloned from yeast during screening of mutants for staurosporine sensitivity [71]. The primary target of staurosporine, a potent inhibitor of PKC, in yeast is the PKC homolog, *PKC1*. However, isolation of additional staurosporine-sensitive strains identified the *STT4* gene which encodes a PtdIns 4-kinase that is different from the *PIK1* gene product. This protein, has a calculated molecular mass of 216 kDa, and shows 25–28% sequence homology in its C-terminal catalytic domain with the corresponding domains of the yeast *PIK1* gene product, the Vps34p, and of the 110 kDa catalytic subunit of mammalian PI 3-kinase. Genetic elimination of *STT4* greatly reduced, but did not abolish, PtdIns 4-kinase activity and (in contrast to *PIK1*) was not lethal, although these strains required osmotic stabilizers for survival. Staurosporine-sensitivity could be suppressed in yeast by overexpression of the PKC homolog, *PKC1*, indicating that Stt4p may be a regulator of PKC-dependent activation pathways in yeast [71]. In subsequent studies, Stt4p was identified as the major target of the PI 3-kinase inhibitor, WT, in *Saccharomyces cerevisiae*. In other yeast strains, this gene was found essential for survival [72]. Interestingly, the product of the *PIK1* gene was found to be insensitive to WT in these studies, in contrast to its mammalian homologs (see below) and its overexpression could not confer WT resistance. On the other hand, overexpression of PtdIns(4)P 5-kinase, and deletion of PLC-1, each resulted in WT resistance, indicating that PtdIns(4,5)P_2 itself has a function more important for this phenotype than those of its hydrolytic products [72]. Very recent studies indicate that Stt4p is required for interorganelle transport of aminophospholipids between the endoplasmic reticulum and the Golgi [73]. Since *STT4* was also identified as a gene capable of complementing a genetic defect causing increased bleomycin sensitivity [74], these data together suggest that Stt4p may be a critical enzyme for cell wall synthesis at the level of phospholipid transport and Golgi function.

It is noteworthy that none of the above enzymes appear to be identical with the 45 and 55 kDa membrane-bound yeast PtdIns 4-kinases [34] which are probably the type II enzymes of yeast that still wait for genetic identification.

4.2. Mammalian PtdIns 4-kinases

Cloning of the first mammalian PtdIns 4-kinase resulted from the detection of hallmarks of the catalytic domain of then known PtdIns kinases in the deduced amino acid sequence of a human expressed sequence tag (EST) clone. This was followed by isolation of clones from a human placenta cDNA library combined with 5′-RACE from cDNA of fetal human brain, and resulted in the cloning of a protein with a calculated molecular mass of 97 kDa [75]. This protein, termed PI4Kα, had strong sequence similarity with the yeast gene, *STT4*, but was only about half its size. Interestingly, both proteins contained a putative pleckstrin-homology (PH) domain that may contribute to their membrane localization. When expressed in Sf9 cells, PI4Kα displayed PtdIns 4-kinase activity that was inhibited by both adenosine and a monoclonal antibody believed to be specific for the type II PtdIns 4-kinase. Based on these criteria, PI4Kα was designated as a type II PtdIns 4-kinase [75].

The expression of two PtdIns 4-kinases in yeast and at least two distinct biochemical activities (the type II and type III enzymes) in mammalian cells justified the search for additional PtdIns 4-kinases. Homology cloning identified two distinct PtdIns 4-

kinases from rat, a 230 kDa enzyme [76], (a homolog of STT4) and a 92 kDa protein similar to PIK1 [77], as well as a human PI4Kβ [78]. In addition, type III PtdIns 4-kinases have also been cloned following the purification of these enzymes from bovine adrenal and brain. As mentioned earlier, two type III PtdIns 4-kinase activities were purified from bovine adrenal, one corresponding to a 110 kDa, and another to a 210 kDa protein [31]. The larger enzyme was also purified from bovine brain [32]. Both of these enzymes were found to be sensitive to the PtdIns 3-kinase inhibitor, WT, although at higher concentrations than inhibit 3-kinases. The cloning of the bovine 110 kDa enzyme, PI4KIIIβ [31], revealed identity to the rat 92 kDa protein and to the human PI4Kβ. The cloned 210 kDa protein, (PI4KIIIα), was identical to the rat 230 kDa PtdIns 4-kinase, and the shorter human PI4Kα appeared to be a splice variant of PI4KIIIα. All of the cloned enzymes (except the human PI4Kα) showed WT sensitivity when expressed in COS-7 cells and were found to be insensitive to adenosine, consistent with their being type III enzymes. The reason why PI4Kα behaves as a type II enzyme [75] remains to be determined (see Table 2 for a list of type III PtdIns 4-kinases).

PI4KIIIβ has two splice variants that affect its primary sequence, one containing an extra 16 amino acid serine-rich cassette C-terminal from the lipid-kinase unique domain. Since this cassette, which is also found in a human PtdIns 4-kinase sequence [79], interrupts a peptide sequence obtained from the isolated protein, we assume that the majority of the expressed enzyme in the adrenal cortex does not contain this sequence. Further splice variants of the PI4KIIIβ transcripts were isolated during 5′-RACE that differ only in their 5′-UTR (T. Balla, unpublished observations, and [79]). This variation, which does not affect the protein sequence in the cow, may be important in the tissue-specific regulation of the expression of these proteins.

Analysis of the tissue distribution of mammalian PtdIns 4-kinases by Northern analysis and in situ hybridization revealed that they are ubiquitously expressed, although PI4KIIIα is expressed predominantly in the brain, while PI4KIIIβ is more widely distributed [31]. Our studies comparing the distribution of PI4KIIIα and PI4KIIIβ in rat brain by in situ hybridization showed that PI4KIIIβ displayed a uniform expression pattern with more pronounced signal in the gray matter, especially in the neurons of the olfactory bulb and the hippocampus including the dentate gyrus. In contrast, while PI4KIIIα was very prominently expressed in neurons of the same structures as PI4KIIIβ, no signal was observed in the white matter nor in the choroid plexus, both of which clearly expressed PI4KIIIβ (A. Zolyomi and T. Balla, in preparation). These data suggest that, with some exceptions, these two enzymes are generally expressed together probably even within the same cells. These findings, together with the above-mentioned genetic studies in yeast and with the high degree of conservation of the two kinases between yeast and mammals, indicate that they must serve non-redundant functions.

It is interesting to note that, in spite of its greater abundance and successful purification from various sources, the type II PtdIns 4-kinase(s) has not yet been cloned. However, we may assume that it will constitute a family of kinases that is significantly different from the type III enzymes that have hitherto been characterized.

5. Cellular processes in which PtdIns 4-kinases are implicated

The following is a short summary of cellular functions in which PtdIns 4-kinases may play a regulatory role. All of the biochemical reactions in cells known to be influenced by 4-phosphorylated inositides require the action of 4-kinases. However, below we will only discuss cellular events in which PtdIns 4-kinases are specifically mentioned or have been studied. In many of these cases, additional phosphorylations of the inositol ring of PtdIns(4)P by 5- and/or 3-kinases are required and research is exploding in determining the structural features that determine the specificity of protein–inositol phospholipid interactions which are mostly, but not exclusively, mediated by protein PH domains. The details of these interactions are discussed in a number of recent reviews [80], and are mentioned in other chapters of this volume.

5.1. Generation of Ins(1,4,5)P_3 and DAG

Originally, PtdIns kinase research was fueled by the notion that such enzymes are necessary for the synthesis of hormone-sensitive PtdIns(4,5)P_2 pools and, hence, the production of Ins(1,4,5)P_3 and DAG. The localization of the most abundant type II PtdIns 4-kinase to the plasma membrane, where receptor-mediated PtdIns(4,5)P_2 hydrolysis takes place, led to the logical assumption that the type II enzyme is involved in the synthesis of hormone-sensitive PtdIns(4,5)P_2 pools. On the other hand, PtdIns kinase activities were also found in other membrane compartments, such as the Golgi [81], secretory granules [82,83] and nuclear membranes [84], but their possible physiological functions in these membrane compartments were not addressed.

The finding that agonist-responsive PtdIns(4,5)P_2 pools are synthesized by WT-sensitive PtdIns 4-kinases, together with the WT-sensitivity of type III, but not of the type II, PtdIns 4-kinases, were the first data to indicate that the type III, rather than type II enzyme(s), are important for this signaling function. Since both of the cloned type III enzymes (α and β) are soluble proteins with no apparent hydrophobic domains, the question arises as to how these enzymes gain access to their membrane-located substrate, PtdIns. Using antibodies against PI4Kα and PI4Kβ, the distribution of these proteins was examined in CHO cells and Hela cells. A 180 kDa endogenous protein reacting with the PI4Kα antibody (presumably the high molecular weight splice variant of PI4Kα), as well as the expressed 97 kDa enzyme were found to be predominantly membrane-bound. In contrast the endogenous (as well as the expressed) 110 kDa PI4Kβ was shown to be present in both soluble and particulate fractions [85]. Cytoimmunofluorescent and confocal microscopy studies in Hela cells using antibodies directed against native or epitope tagged enzymes, suggested that PI4Kα (both endogenous and expressed) was localized to the endoplasmic reticulum (ER), while PI4Kβ was associated with Golgi membranes [85]. On the other hand, the rat 230 and 97 kDa enzymes (equivalents of a long PI4Kα and PI4Kβ, respectively) were both localized to the Golgi when expressed in COS-7 cells [76,77]. Our studies with the expressed bovine enzymes (PI4KIIIα and PI4KIIIβ) confirmed the predominantly membrane-localization of the α enzyme as opposed to the mostly soluble β form (unpublished observations). We also found that PI4KIIIα enzyme was present in large amounts in a Triton-insoluble, presumably cytoskeletal compartment, a finding consistent with that described for the human enzyme [85].

Although the EGF receptor has been shown to associate with and activate the type II PtdIns 4-kinase in several studies [21,46], our finding that the EGF-stimulated increase of labeled PtdIns(4)P in COS-1 cells is inhibited by high concentrations of WT suggests the involvement of type III PtdIns 4-kinases (Kim, Downing and Balla, manuscript in preparation). The mechanisms by which WT-sensitive PtdIns 4-kinases are activated by G-protein-coupled receptors and receptor tyrosine kinases has yet to be determined.

5.2. PtdIns 4-kinases and Golgi function

As mentioned above, PtdIns 4-kinase activity has been found to be associated with Golgi membranes [81], and expression studies indicate that some of the PI4Kβ (and possibly the 230 kDa α enzyme) localizes to the Golgi apparatus [76,85]. The importance of inositol phospholipids in Golgi function has also been firmly established, although the details of this regulation are only now beginning to unfold. Members of the ARF family of small GTPases are critical for the membrane budding–fusion events associated with Golgi function [86]. ARF proteins are key regulators of the association of coat proteins with donor membranes in the Golgi and are also recognized as activators of phospholipase D activity, the latter being important in the generation of phosphatidic acid from phosphatidylcholine. PtdIns(4,5)P_2 has been shown to be an important regulator of ARF1 protein function in several ways: it was reported to promote GDP dissociation from ARF [87], and also to activate ARF GAP [88]. More recently, PtdIns(3,4,5)P_3 was found to be a regulator of guanine nucleotide exchange on ARF [89], acting via an exchange factor, ARNO, which contains a PtdIns(3,4,5)P_3 binding PH domain, in addition to its guanine nucleotide exchange promoting Sec7 domain [90]. The ability of ARF proteins to activate PLD activity is also highly dependent on

PtdIns(4,5)P_2 [91], and one of the most potent endogenous PLD 'inhibitors' has proven to be a PtdIns(4,5)P_2 (or PtdIns(3,4,5)P_3) 5-phosphatase enzyme [92], also known as synaptojanin [93].

All of these data suggest that 4-phosphorylation of PtdIns (along with 5 and possibly 3-phosphorylations, see the articles by K.A. Hinchliffe et al., and M.P. Wymann and L. Pirola, this special issue) is an important step in the regulation of coating and uncoating of Golgi membranes. The question that remains to be answered is whether the 4-kinase that is involved is actively regulated as part of the regulatory process. The only available data addressing this question was a report on the ARF-activation of PLD activity in permeabilized U938 cells [94]. In this system, it was found that GTPγS-activated (presumably ARF-mediated) PLD activity was greatly potentiated by ATP and that this potentiation was reversed by a blocking antibody (4C5G) that specifically inhibits the type II PtdIns 4-kinase, as well as by neomycin that is known to bind to (and mask the actions of) PtdIns(4,5)P_2 [94]. Since a blocking antibody against PI 3-kinase had no effect, it was concluded that the ATP-dependent regulatory step was required to synthesize PtdIns(4,5)P_2 by the type II PtdIns 4-kinase and additional 5-kinase enzymes. While these data clearly suggest the involvement of type II kinase, it is important to note that the same antibody was found to inhibit expressed PI4Kα, which is a splice variant of a type III PtdIns 4-kinase [75]. Clearly, more data are required to establish identity of the PtdIns 4-kinase involved in the complex machinery controling Golgi function.

5.3. PtdIns 4-kinases and the secretory pathway

Another process in which inositol phospholipids and PtdIns kinases are implicated is the release of vesicles from secretory cells and perhaps, in a similar manner, the release of presynaptic vesicles. Again, it was found in early studies that membrane fractions enriched in secretory vesicles contain PtdIns 4-kinase activity [82,95]. The kinase present in bovine adrenal chromaffin granules was inhibited by low (10^{-7} M) concentrations of Ca^{2+}, and had a low K_m for ATP (40–60 μM) which are characteristics of type II PtdIns 4-kinases [82]. Functional studies on regulated exocytosis in various cells suggested that inositol lipids and their phosphorylation are critical to the maintenance of the secretory response. Based on studies in permeabilized adrenal chromaffin cells, where manipulation of phosphoinositide levels greatly affected Ca^{2+}-dependent secretion, it was postulated that PtdIns(4,5)P_2 and not its hydrolytic products are important for exocytosis [96]. In permeabilized PC12 cells, it has been shown that an ATP-dependent priming step is necessary for optimal Ca^{2+}-induced exocytosis [97]. This ATP-dependent priming is gradually lost after permeabilization due to the loss of proteins that presumably mediate this process. Reconstitution studies identified three activities with molecular sizes of ~500, 120 and 20 kDa that were able to restore priming, the smallest of which was identified as PtdIns transfer protein [97]. Mutations in *SEC14*, the gene encoding the yeast homolog of this protein was already known to cause defects in constitutive secretion in yeast [98]. While PtdIns transfer protein is important in supplying PtdIns, and is required for PLC activity in permeabilized HL60 cells [99], its function does not require ATP. Together with the importance of PtdIns(4,5)P_2, it was therefore plausible to assume that ATP was needed for PtdIns phosphorylation, and indeed, the high molecular mass complex with priming activity was shown to contain the type I PtdIns(4)P 5-kinase enzyme [100]. Therefore, the only missing link in this process is the PtdIns 4-kinase enzyme required for the series of phosphorylation reactions.

One can only speculate about the nature of the putative PtdIns 4-kinase that participates in the priming process. High ATP concentrations (EC_{50} ~0.5 mM) were needed to maintain both PtdIns(4,5)P_2 levels and the secretory response in permeabilized bovine chromaffin cells [96], consistent with the involvement of a type III, rather than a type II, PtdIns 4-kinase. Also, the molecular mass of one of the priming activities was found to be 120 kDa, close to that of the type III PI4Kβ [31]. WT, at relatively high concentrations (above μM) was found to inhibit the sustained, but not the initial phase of secretion in several secretory cell types [101,102], which corresponds to the ATP-dependent phase of exocytosis of permeabilized cells. Although the possible role of myosin light chain kinase cannot be ruled out as the target of WT in those studies, these findings were also consistent with the putative role of WT-sensi-

tive, type III enzyme(s). In contrast, priming was found to be insensitive to 1 μM WT, a concentration that should (at least partially) inhibit type III PtdIns 4-kinases, which argues against the role of a type III enzymes [103]. A recent report has indicated the involvement of a PtdIns 4-kinase in exocytosis of bovine adrenal chromaffin cells, and found the PtdIns 4-kinase associated with chromaffin granules to be sensitive to inhibition by either quercetin or phenylarsine-oxide [83]. The latter inhibitory effect was reversible by 2,3-dimercaptopropanol, but not by β-mercaptoethanol. Moreover, it was recently proposed that this membrane-associated PtdIns 4-kinase is regulated by a G_o/Rho-dependent mechanism in chromaffin granules [104].

An increasing number of proteins that are involved in neurotransmitter release and the concomitant membrane internalization events in presynaptic membranes have been found to bind PtdIns(4,5)P_2 and other PtdIns- and inositol-polyphosphates. Among these are the proteins, synaptotagmin and the GTPase, dynamin [105]. Moreover, one of the proteins that associates with amphiphysin, an SH3 domain containing protein of synaptic vesicles (that also recruits dynamin to these membranes), is synaptojanin, a type II 5-phosphatase, that can dephosphorylate PtdIns(4,5)P_2 as well as PtdIns(3,4,5)P_3 [93]. Therefore, the enzymes that synthesize PtdIns(4,5)P_2 on the surface of synaptic vesicles and the inner leaflet of presynaptic membranes will also be of great interest in studies of the regulation of neurotransmitter release. No data are as yet available as to the nature of PtdIns 4-kinase(s) that functions in this context.

5.4. PtdIns 4-kinases and cytoskeleton

Association of PtdIns 4-kinase (as well as other enzymes of the PtdIns cycle) with the actin-cytoskeleton was found in EGF-stimulated A431 cells [45] and in thrombin-stimulated platelets [106]. It has also been shown that the EGF-sensitive pool of PtdIns(4,5)P_2 is mostly present in glycophospholipid-rich, triton-insoluble membrane compartments that also contain caveolin [107,108]. Type II PtdIns 4-kinase was found to associate with $\alpha_3\beta_1$ integrin via transmembrane 4 receptor family members, CD63 and CD81 [109], and this complex was predominantly membrane-localized and not present in focal adhesions, another assembly point of many signaling complexes. The connection between certain small G-proteins (such as Rac or cdc42), PI 3-kinases, and the formation of membrane-ruffles which requires active participation of cytoskeletal proteins has been proposed (see the article by M.P. Wymann and L. Pirola, this special issue). The interaction between PtdIns(4,5)P_2 and proteins that regulate actin polymerization is also well established. Profilin and gelsolin are two proteins that bind PtdIns(4,5)P_2, and also bind to monomeric or the barbed end of polymeric actin, respectively, in the absence of the phospholipid (reviewed in [12]). Profilin also has a profound inhibitory effect on phospholipase C-γ, which could be overcome by tyrosine phosphorylation of the latter [110]. Changes in the level of PtdIns(4,5)P_2 either by PLC activation or by the action of PtdIns kinases could therefore greatly affect the actin cytoskeleton as suggested in numerous studies. More recently, the 110 kDa type III PtdIns 4-kinase β has been implicated in the endocytosis of muscarinic cholinergic receptors. High concentrations of WT, LY-294002, and 20 μM phenylarsine oxide, each inhibiting the [^{32}P]phosphate-labeling of PtdIns(4)P in SH-SY5Y neuroblastoma cells, also inhibited the agonist-dependent receptor endocytosis [111]. This study also demonstrated that PI4Kβ, but not PI4Kα, was enriched in these cells in membrane fractions enriched in endocytotic vesicles. The putative effects of PI4Kβ on receptor endocytosis were not mediated by changes in cytoskeleton, since inhibitors affecting the latter had no effects on receptor endocytosis. The exact mechanism by which PI4Kβ affects muscarinic receptor internalization and whether this also applies to other GPCRs is yet to be determined.

5.5. PtdIns 4-kinase and nuclear functions

Almost all the elements of the classical phosphoinositide cycle have been shown to be present in the nucleus. Since during cell fractionation significant plasma membrane contamination of the nuclear fraction can occur, several thorough studies have been performed to address the real presence and possible functions of nuclear inositides. PtdIns 4-kinase (also PLC, PtdIns(4)P 5-kinase and DG kinase) activity was found to be present in the nuclear matrix of

rat liver and NIH 3T3 cells after nuclease treatment and high salt extraction. By selective extraction, the PtdIns 4-kinase was localized to the peripheral matrix (lamina–pore complex) [112]. Although it was not tested whether the kinase was a type II or type III activity (it was producing PtdIns 4-phosphate), this finding is even more remarkable in light of the cloning of the yeast *PIK1* as a protein that is part of the nuclear pore complex [68]. It is not clear if the nuclear PtdIns 4-kinase is regulated by an agonist ligand or participates in the regulation of any known nuclear function. However, one of the DNA polymerases, the low specific activity DNA polymerase α, was found to be activated selectively by PtdIns(4)P and its hydrolytic product, Ins(1,4)P_2 [113]. Whether this activation has any physiological relevance remains to be determined. Agonist-induced changes in nuclear inositides have been reported in Swiss 3T3 cells, where IGF-I was found to cause a decrease of nuclear PtdIns(4)P and PtdIns(4,5)P_2, but bombesin which caused a large decrease of the same lipids in the plasma membrane, did not induce a similar change in the nucleus [114]. There are several reports that Ins(1,4,5)P_3 receptors exist in both the outer and inner nuclear membrane and that the Ca^{2+} concentration inside the nuclear membrane (as a continuation of the luminal space of the ER) has noticeable effects on the morphology of the nuclear pore [115]. Hence, evidence is accumulating that nuclear inositides and the kinases that synthesize them are worthy candidates for regulation of nuclear functions, but more studies are needed to better define the processes that are controlled by them.

6. Future directions

Based on advances of the last 10 years, phosphoinositide research has now entered its second blooming. Following exploration of the details of receptor-stimulated phosphoinositide turnover and its second messenger functions, there is renewed interest in phosphoinositides as potential membrane docking sites and regulators of various signaling proteins. Understanding the molecular basis of inositol lipid–protein interactions and their importance in controlling various intracellular signaling cascades will certainly represent a major goal of future studies. An already emerging question is whether the precursor function and the anchoring and regulatory functions of phosphoinositides are separable or overlapping. Recent data from our laboratory indicate that the majority of PtdIns(4,5)P_2 that interacts with protein PH domains may be under separate regulatory control than the PtdIns(4,5)P_2 that serves as precursor for Ins(1,4,5)P_3 to release Ca^{2+}. One of the key differences between these functionally separable pools is the PtdIns 4-kinase that controls their synthesis (P. Varnai and T. Balla, submitted). These data imply that isolation and cloning of additional PtdIns 4-kinases (perhaps those of the type II family) will be a top priority for studies on phosphoinositide–protein interactions. Similarly, the identification of inhibitors that can discriminate between type II and type III PtdIns 4-kinases and between 3- and 4-kinases will greatly facilitate research in this direction.

Another closely related question is the compartmentalization of these reactions. From the pleiotropic effects of inositides in the various regulatory processes linked to different cellular organelles, it is obvious that these reactions need to be confined to very restricted membrane compartments even within a particular membrane type. For example, recent data suggest that the receptor-mediated changes in inositide metabolism and the enzymes that participate in these reactions are found in membrane clusters [116] and in caveolin-enriched membrane fractions [107]. Therefore, there is a need to resolve these biochemical events to the subcellular level, and possibly to image them as they happen in real time in the living cell. Several such ingenious attempts have begun to appear in the literature [116–118] and they will surely represent a powerful approach to understand inositide-based cell regulation.

Although many extremely useful data have been published showing stereospecific association of various regulatory proteins with inositol lipids (and the impact of those interactions on the function of the protein) in vitro, the next question is to determine whether these interactions, and their functional consequences, do actually happen in cells in vivo. There is an increasing need to establish methodology that allows these reactions to be studied within living cells to complement in vitro biochemical data. One of the best examples is the case of PtdIns 4-kinases, whose activity is usually measured by in vitro assays using

artificial substrates. There is already important evidence showing that the ability of a PtdIns molecule to be phosphorylated by a kinase may largely depend on the presence of other cellular membrane components. For example, the phosphatidylinositol transfer protein, PI-TP, has been shown to greatly influence the 'activity' of PtdIns 4-kinases [49] as well as of a PI 3-kinase [119]. Also, the effect of tyrosine phosphorylation of PLC-γ on its catalytic activity was greatly enhanced by the presence of profilin in the kinase assay [110]. These data also show that a better understanding of the regulation of PtdIns 4-kinases will require the combination of various approaches, including those that preserve the molecular interactions present in intact cells.

Another important question is whether the PtdIns 4-kinases also possess protein kinase activities that could be relevant to their function(s). A whole group of proteins, termed PI kinase homologs, have been described based on sequence homologies within their kinase domains with the known PI 3- and 4-kinases. Almost all of these proteins are of high molecular weight (over 200 kDa), and are involved in cell cycle control [120]. However, in spite of their similarity in their catalytic domains with the PtdIns kinases, these proteins are believed to be exclusively protein kinases, and do not possess lipid kinase activities. Conversely, PI 3-kinase have been shown to phosphorylate its own p85 regulatory subunit, causing a major loss of the PI 3-kinase activity of the complex [121]. There are intriguing questions about the structural determinants that are responsible for an enzyme's ability to phosphorylate PtdIns as opposed to proteins and whether protein substrates for PtdIns 4-kinases can be identified. Interestingly, one of the serine-threonine kinases, phosphorylase B kinase, has been found to phosphorylate PtdIns [122], but it is not known if this reaction occurs within cells. Nonetheless, the evolutionary link between PtdIns kinases and the PI-kinase related kinases is an exciting area of research that deserves special attention.

Finally, there appears to be an emerging scheme in the research field of inositide-based membrane assembly and disassembly processes: the basic participating elements include some form of a GTPase, with its guanine nucleotide exchange factor, or its GTPase activators (GAPs), and the enzymes producing or degrading the inositol phospholipid at the membrane. Depending on the membrane and the downstream effectors, the name of the participants will vary as will the phosphoinositide that is involved. The PH domain [80] present in these regulatory proteins is the prime candidate to determine the specific inositide requirement of the process and confers lipid regulation. This, of course, is not the only way phosphoinositides operate via protein effectors, since several in vitro examples are known where serine-threonine kinases, such as PKC [123] or PKB [124], are affected by inositides. It is a major challenge of the near future to identify the particular phosphoinositide kinase enzymes that serve as regulators of the assembly and activity of signaling complexes in the context of each specific cellular process in a defined membrane compartment. This will allow understanding of the pleiotropic actions of inositides and contribute to their definition as potential therapeutic targets.

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References

[1] R.J. Anderson, E.G. Roberts, J. Biol. Chem. 89 (1930) 611–617.
[2] C.E. Ballou, Y.C. Lee, Biochemistry 3 (1964) 682–685.
[3] J. Folch, D.W. Wooley, J. Biol. Chem. 142 (1942) 963–964.
[4] E. Klenk, R. Sakai, Hoppe-Zeyl. Z. Physiol. Chem. 258 (1939) 33–38.
[5] J. Folch, J. Biol. Chem. 177 (1949) 497–504.
[6] J. Folch, F.N. LeBaron, Can. J. Physiol. Biochem. 34 (1956) 305–319.
[7] M.R. Hokin, L.E. Hokin, J. Biol. Chem. 203 (1953) 967–977.
[8] R.H. Michell, Biochim. Biophys. Acta 415 (1975) 81–147.
[9] J.A. Creba, C.P. Downes, P.T. Hawkins, G. Brewster, R.H. Michell, C.J. Kirk, Biochem. J. 212 (1983) 733–747.
[10] M.J. Berridge, Biochem. J. 212 (1983) 849–858.

[11] H. Streb, R.F. Irvine, M.J. Berridge, I. Schulz, Nature 306 (1983) 67–68.
[12] L.J. Pike, Endocr. Rev. 13 (1992) 692–706.
[13] C.L. Carpenter, L.C. Cantley, Biochemistry 29 (1990) 11147–11156.
[14] R.H. Michell, J.L. Harwood, R. Coleman, J.N. Hawthorne, Biochim. Biophys. Acta 144 (1967) 649–658.
[15] L.E. Hokin, M.R. Hokin, Biochim. Biophys. Acta 84 (1964) 563–575.
[16] J.L. Harwood, J.N. Hawthorne, Biochim. Biophys. Acta 171 (1969) 75–88.
[17] A. Graziani, L.E. Ling, G. Endemann, C.L. Carpenter, L.C. Cantley, Biochem. J. 284 (1992) 39–45.
[18] R. Wetzker, R. Klinger, J. Hsuan, M.J. Fry, A. Kauffmann-Zeh, E. Muller, H. Frunder, M.D. Waterfield, Eur. J. Biochem. 200 (1991) 179–185.
[19] W.-M. Hou, Z.-L. Zhang, H.H. Tai, Biochim. Biophys. Acta 959 (1988) 67–75.
[20] F.D. Porter, Y.-S. Li, T.F. Deuel, J. Biol. Chem. 263 (1988) 8989–8995.
[21] D.H. Walker, N. Dougherty, L.J. Pike, Biochemistry 27 (1988) 6504–6511.
[22] G. Endemann, S.N. Dunn, L.C. Cantley, Biochemistry 26 (1987) 6845–6852.
[23] M. Whitman, D. Kaplan, T. Roberts, L. Cantley, Biochem. J. 247 (1987) 165–174.
[24] M. Whitman, C.P. Downes, M. Keller, T. Keller, L.C. Cantley, Nature 332 (1988) 644–646.
[25] G.C. Endemann, A. Graziani, L.C. Cantley, Biochem. J. 273 (1991) 63–66.
[26] Y.-S. Li, F.D. Porter, R.M. Hoffman, T.F. Deuel, Biochem. Biophys. Res. Commun. 160 (1989) 202–209.
[27] A. Yamakawa, T. Takenawa, J. Biol. Chem. 263 (1988) 17555–17560.
[28] A. Yamakawa, M. Nishizawa, K.T. Fujiwara, S. Kawai, H. Kawasaki, K. Suzuki, T. Takenawa, J. Biol. Chem. 266 (1991) 17580–17583.
[29] S. Nakanishi, K.J. Catt, T. Balla, Proc. Natl. Acad. Sci. USA 92 (1995) 5317–5321.
[30] G.J. Downing, S. Kim, S. Nakanishi, K.J. Catt, T. Balla, Biochemistry 35 (1996) 3587–3594.
[31] T. Balla, G.J. Downing, H. Jaffe, S. Kim, A. Zolyomi, K.J. Catt, J. Biol. Chem. 272 (1997) 18358–18366.
[32] T. Gehrmann, G. Vereb, M. Schmidt, D. Klix, E.H. Meyer, M. Varsanyi, L.M.G. Heilmeyer Jr., Biochim. Biophys. Acta 1311 (1996) 53–63.
[33] A.R. Saltiel, J.A. Fox, P. Sherline, N. Sahyoun, P. Cuatrecasas, Biochem. J. 241 (1987) 759–763.
[34] G.M. Carman, R.J. Buxeda, J.T. Nickels Jr., Adv. Lipobiol. 1 (1996) 367–385.
[35] J.T. Nickels Jr., R.J. Buxeda, G.M. Carman, J. Biol. Chem. 269 (1994) 11018–11024.
[36] R.J. Buxeda, J.T. Nickels Jr., G.M. Carman, J. Biol. Chem. 268 (1993) 6248–6255.
[37] K. Koreh, M.E. Monaco, J. Biol. Chem. 261 (1986) 88–91.
[38] R.W. Dougherty, P.P. Godfrey, P.C. Hoyle, J.W. Putney Jr., R.J. Freer, Biochem. J. 222 (1984) 307–314.
[39] T. Balla, A.J. Baukal, G. Guillemette, K.J. Catt, J. Biol. Chem. 263 (1988) 4083–4091.
[40] P. Enyedi, B. Büki, I. Mucsi, A. Spät, Mol. Cell. Endocrinol. 41 (1985) 105–112.
[41] L.R. Stephens, T.R. Jackson, P.T. Hawkins, Biochem. J. 296 (1993) 481–488.
[42] H. Arai, J.A. Escobedo, Mol. Pharmacol. 50 (1996) 522–528.
[43] L.J. Pike, A.T. Eakes, J. Biol. Chem. 262 (1987) 1644–1651.
[44] D.H. Walker, L.J. Pike, Proc. Natl. Acad. Sci. USA 84 (1987) 7513–7517.
[45] B. Payrastre, P.M.P. van Bergen en Henegouwen, M. Breton, J.C. den Hartigh, M. Plantavid, A.J. Verkleij, J. Boonstra, J. Cell Biol. 115 (1991) 121–128.
[46] C. Cochet, O. Filhol, B. Payrastre, T. Hunter, G.N. Gill, J. Biol. Chem. 266 (1990) 637–644.
[47] A. Kauffmann-Zeh, R. Klinger, G. Endemann, M.D. Waterfield, R. Wetzker, J.J. Hsuan, J. Biol. Chem. 269 (1994) 31243–31251.
[48] P. Pertile, L.C. Cantley, Biochim. Biophys. Acta 1248 (1995) 129–134.
[49] A. Kauffmann-Zeh, G.M. Thomas, A. Ball, S. Prosser, E. Cunningham, S. Cockcroft, J.J. Hsuan, Science 268 (1995) 1188–1190.
[50] S.B. Chahwala, L.F. Fleischman, L. Cantley, Biochemistry 26 (1987) 612–622.
[51] L.D. Chong, A. Traynor-Kaplan, G.M. Bokoch, M.A. Schwartz, Cell 79 (1994) 507–513.
[52] T.N. Lo, S.P. Eng, L.A. Jaseph, M.A. Beaven, C.S. Lo, Biochim. Biophys. Acta 970 (1988) 51–60.
[53] D.H. Walker, L.J. Pike, Biochim. Biophys. Acta 1055 (1990) 295–298.
[54] S.P. Eng, C.S. Lo, Life Sci. 46 (1990) 273–279.
[55] W. Yang, W.F. Boss, Arch. Biochem. Biophys. 313 (1994) 112–119.
[56] S. Vogel, J. Hoppe, Eur. J. Biochem. 154 (1986) 253–257.
[57] G.A. Lundberg, R. Sundler, B. Jergil, Biochim. Biophys. Acta 922 (1987) 1–7.
[58] H. Kato, I. Uno, T. Ishikawa, T. Takenawa, J. Biol. Chem. 264 (1989) 3116–3121.
[59] K.M. Holland, M.J. Homann, C.J. Belunis, G.M. Carman, J. Bacteriol. 170 (1988) 828–833.
[60] A. Enyedi, A. Farago, B. Sarkadi, I. Szasz, G. Gardos, FEBS Lett. 161 (1983) 158–162.
[61] A. Coco, I.G. Macara, Am. J. Physiol. 251 (1986) C883–C886.
[62] V.D. Bianca, P. De Togni, M. Grzeskowiak, L.M. Vicentini, F. De Virgilio, Biochim. Biophys. Acta 886 (1986) 441–447.
[63] H. Yano, S. Nakanishi, K. Kimura, N. Hanai, Y. Saitoh, Y. Fukui, Y. Nonomura, Y. Matsuda, J. Biol. Chem. 268 (1993) 25846–25856.
[64] A. Arcaro, M.P. Wymann, Biochem. J. 296 (1993) 297–301.
[65] R.A. Currie, B.M.G. MacLeod, C.P. Downes, Curr. Biol. 7 (1997) 184–190.

[66] C.A. Flanagan, E.A. Schnieders, A.W. Emerick, R. Kunisawa, A. Admon, J. Thorner, Science 262 (1993) 1444–1448.
[67] C.A. Flanagan, J. Thorner, J. Biol. Chem. 267 (1992) 24117–24125.
[68] J.F. Garcia-Bustos, F. Marini, I. Stevenson, C. Frei, M.N. Hall, EMBO J. 13 (1994) 2352–2361.
[69] I.D. Hiles, M. Otsu, S. Volinia, M.J. Fry, I. Gout, R. Dhand, G. Panayotou, F. Ruiz-Larrea, A. Thompson, N.F. Totty, J.J. Hsuan, S.A. Courtneidge, P.J. Parker, M.D. Waterfield, Cell 70 (1992) 419–429.
[70] J.H. Stack, S.D. Emr, J. Biol. Chem. 269 (1994) 31552–31562.
[71] S. Yoshida, Y. Ohya, M. Goebl, A. Nakano, Y. Anrakur, J. Biol. Chem. 269 (1994) 1166–1171.
[72] N.S. Cutler, J. Heitman, M.E. Cardenas, J. Biol. Chem. 272 (1997) 27671–27677.
[73] P.J. Trotter, W.-I. Wu, J. Pedretti, R. Yates, D.R. Voelker, J. Biol. Chem. 273 (1998) 13189–13196.
[74] A. Pramanik, E. Garcia, R. Ospina, M. Powell, M. Martinez, W. Alejo, J. McCoy, C.W. Moore, Cell. Mol. Biol. 43 (1997) 1007–1018.
[75] K. Wong, L.C. Cantley, J. Biol. Chem. 269 (1994) 28878–28884.
[76] T. Nakagawa, K. Goto, H. Kondo, J. Biol. Chem. 271 (1996) 12088–12094.
[77] T. Nakagawa, K. Goto, H. Kondo, Biochem. J. 320 (1996) 643–649.
[78] R. Meyers, L.C. Cantley, J. Biol. Chem. 272 (1997) 4384–4390.
[79] K. Suzuki, H. Hirano, K. Okutomi, M. Suzuki, Y. Kuga, T. Fujiwara, N. Kanemoto, K. Isono, M. Horie, DNA Res. 4 (1997) 273–280.
[80] M.A. Lemmon, M. Falasca, K.M. Ferguson, J. Schlessinger, Trends Cell Biol. 7 (1997) 237–242.
[81] B. Jergil, R. Sundler, J. Biol. Chem. 258 (1983) 7968–7973.
[82] E.S. Husebye, T. Flatmark, Biochim. Biophys. Acta 968 (1988) 261–265.
[83] C. Wiedemann, T. Schäfer, M.M. Burger, EMBO J. 15 (1996) 2094–2101.
[84] C.D. Smith, W.W. Wells, J. Biol. Chem. 258 (1983) 9368–9373.
[85] K. Wong, R. Meyers, L.C. Cantley, J. Biol. Chem. 272 (1997) 13236–13241.
[86] R.A. Kahn, J.K. Yucel, V. Malhotra, Cell 75 (1993) 1045–1048.
[87] T. Terui, R.A. Kahn, P.A. Randazzo, J. Biol. Chem. 269 (1994) 28130–28135.
[88] P.A. Randazzo, R.A. Kahn, J. Biol. Chem. 269 (1994) 10758–10763.
[89] J.K. Klarlund, L.E. Rameh, L.C. Cantley, J.M. Buxton, J.J. Holik, C. Sakelis, V. Patki, S. Corvera, M.P. Czech, J. Biol. Chem. 273 (1998) 1859–1862.
[90] J.K. Klarlund, A. Guilherme, J.J. Holik, J.V. Virbasius, A. Chawla, M.P. Czech, Science 275 (1997) 1927–1930.
[91] M. Liscovitch, V. Chalifa, P. Pertile, C.-S. Chen, L.C. Cantley, J. Biol. Chem. 269 (1994) 21403–21406.
[92] J.S. Han, J.K. Chung, H.S. Kang, J. Donaldson, Y.S. Bae, S.G. Rhee, J. Biol. Chem. 271 (1996) 11163–11169.
[93] P.S. McPherson, E.P. Garcia, V.I. Slepnev, C. David, X. Zhang, D. Grabs, W.S. Sossin, R. Bauerfeind, Y. Nemoto, P. De Camilli, Nature 379 (1996) 353–357.
[94] P. Pertile, M. Liscovitch, V. Chalifa, L.C. Cantley, J. Biol. Chem. 270 (1995) 5130–5135.
[95] J.T. Buckley, Y.A. Lefebvre, J.N. Hawthorne, Biochim. Biophys. Acta 239 (1971) 517–519.
[96] D.A. Eberhard, C.L. Cooper, M.G. Low, R.W. Holz, Biochem. J. 268 (1990) 15–25.
[97] J.C. Hay, T.F.J. Martin, Nature 366 (1993) 572–575.
[98] V.A. Bankaitis, D.E. Malehorn, S.D. Emr, R. Greene, J. Cell Biol. 108 (1989) 1271–1281.
[99] G.M.H. Thomas, E. Cunningham, A. Fensome, A. Ball, N.F. Totty, O. Truong, J.J. Hsuan, S. Cockcroft, Cell 74 (1993) 919–928.
[100] J.C. Hay, P.L. Fisette, G.H. Jenkins, K. Fukami, T. Takenawa, R.A. Anderson, T.F.J. Martin, Nature 374 (1995) 173–177.
[101] M. Oharaimaizumi, T. Sakurai, S. Nakamura, S. Nakanishi, Y. Matsuda, S. Muramatsu, Y. Nonomura, K. Kurokawa, Biochem. Biophys. Res. Commun. 185 (1992) 1016–1021.
[102] K. Kumakura, K. Sasaki, T. Sakurai, M. Ohara-Imaizumi, H. Misonou, S. Nakamura, Y. Matsuda, Y. Nonomura, J. Neurosci. 14 (1994) 7695–7703.
[103] T.F.J. Martin, K.M. Loyet, V.A. Barry, J.A. Kowalchik, Biochem. Soc. Trans. 25 (1997) 1137–1141.
[104] S. Gasman, S. Chasserot-Golaz, P. Hubert, D. Aunis, M.F. Bader, J. Biol. Chem. 273 (1998) 16913–16920.
[105] P. De Camilli, K. Takei, Neuron 16 (1996) 481–486.
[106] P. Grondin, M. Plantavid, C. Sultan, M. Breton, G. Mauco, H. Chap, J. Biol. Chem. 266 (1991) 15705–15709.
[107] L.J. Pike, L. Casey, J. Biol. Chem. 271 (1996) 26453–26456.
[108] M.G. Waugh, D. Lawson, S.K. Tan, J.J. Hsuan, J. Biol. Chem. 273 (1998) 17115–17121.
[109] F. Berditchevski, K.F. Tolias, K. Wong, C.L. Carpenter, M.E. Hemler, J. Biol. Chem. 272 (1997) 2595–2598.
[110] P.J. Goldschmidt-Clermont, J.W. Kim, L.M. Machesky, S.G. Rhee, T.D. Pollard, Science 251 (1991) 1231–1233.
[111] S.D. Sorensen, D.A. Linseman, E.L. McEwen, A.M. Heacock, S.K. Fisher, Mol. Pharmacol. 53 (1998) 827–836.
[112] B. Payrastre, M. Nievers, J. Boonstra, M. Breton, A.J. Verkleij, P.M.P. VanBergen en Henegouwen, J. Biol. Chem. 267 (1992) 5078–5084.
[113] V. Sylvia, G. Curtin, J. Norman, J. Stec, D. Busbee, Cell 54 (1988) 651–658.
[114] N. Divecha, H. Banfic, R.F. Irvine, EMBO J. 10 (1991) 3207–3214.
[115] C. Perez-Terzic, J. Pyle, M. Jaconi, L. Stehno-Bittel, D. Clapham, Science 273 (1996) 1875–1877.
[116] T.P. Stauffer, T. Meyer, J. Cell Biol. 139 (1997) 1447–1454.
[117] T.P. Stauffer, S. Ahn, T. Meyer, Curr. Biol. 8 (1998) 343–346.

[118] K. Venkateswarlu, P.B. Oatey, J.M. Tavare, P.J. Cullen, Curr. Biol. 8 (1998) 463–466.
[119] C. Panaretou, J. Domin, S. Cockcroft, M.D. Waterfield, J. Biol. Chem. 272 (1997) 2477–2485.
[120] T. Hunter, Cell 83 (1995) 1–4.
[121] R. Dhand, I. Hiles, G. Panayotou, S. Roche, M.J. Fry, I. Gout, N.F. Totty, O. Truong, P. Vicendo, K. Yonezawa, M. Kasuga, S.A. Courtneidge, M.D. Waterfield, EMBO J. 13 (1994) 522–533.
[122] Z. Georgoussi, L.M.G. Heilmeyer Jr., Biochemistry 25 (1986) 3867–3874.
[123] A. Toker, M. Meyer, K.K. Reddy, J.R. Falck, R. Aneja, S. Aneja, A. Parra, D.J. Burns, L.M. Ballas, L.C. Cantley, J. Biol. Chem. 269 (1994) 32358–32367.
[124] T.F. Franke, D.R. Kaplan, L.C. Cantley, A. Toker, Science 275 (1997) 665–668.
[125] K. Zhou, K. Takegawa, S.D. Emr, R.A. Firtel, Mol. Cell. Biol. 15 (1995) 5645–5656.